REMARKS

This paper is filed in Response to the Office Action mailed June 28, 2011, and in connection with interviews held with Examiner Bristol on June 30, and September 22, and 2011. Claim 35 has been cancelled without prejudice. Applicants maintain the right to prosecute the cancelled claims in any related application claiming the benefit of priority of the subject application. Accordingly, claims 27 to 32, 34, 36 to 43 and 48 to 59 are under consideration on the merits.

Applicants' representative wishes to thank the Examiner for the courtesies extended in granting the interviews during which all grounds for rejection were discussed. Applicants believe that the amended claims accurately reflect the discussion.

The Claim Amendments

The claim amendments are supported throughout the specification. In particular, the amendment to claims 27 to 29, 42, 43, and 53 to 58 to recite "apolipoprotein B containing LDL" and "apolipoprotein B containing oxLDL" is supported, for example, at page 13, last paragraph, to page 14, second paragraph, which discloses anti-apoB antibody and SAM-6 binding to LDL and oxLDL, which contains apolipoprotein B (see, also, page 2, second paragraph, page 3, last paragraph, and Figure 6). The amendments to revise or insert the percent (%) identity to a reference sequence, namely SEQ ID NOs.:1 or 3, is supported, for example, at page 9, second paragraph. The remaining amendments were made to improve clarity or to correct typographical errors. Thus, as the claim amendments are supported throughout the originally filed specification or were made to address informalities, no new matter has been added and entry thereof is respectfully requested.

Previously Filed Exhibit C

Attached herewith for the Examiner's convenience are selected pages from PCT WO 2010/088739, which were previously submitted in part with a complete copy of WO 2010/088739 on April 15, 2011. As previously noted, PCT WO 2010/088739 discloses, among other things, sequences of variant antibodies, antigen binding activities, and studies of antibodies, including variant antibodies, and VH chain alone, binding to target antigen that SAM-6 antibody binds (Examples 13-19). Notably, the attached include a description of binding studies demonstrating that SAM-6.10 (aka SAM-6) scFv, sequence variants and a heavy chain variable region sequence alone (VH alone, SEQ ID NO:3) without a light chain

variable region sequence, have binding activity for apolipoprotein B100, LDL, VLDL and/or deglycosylated LDL (Examples 13 and 19). The different SAM-6 VH and VL chain sequences employed in the binding studies are shown, and the changes made to VH sequences summarized, in pages 87 to 91, 94 and 95 of WO 2010/088739, which are attached for the Examiner's convenience.

In particular, for example, ELISA analysis revealed that SAM-6 scFV and an scFV SAM-6 sequence variant, denoted SAM-6 1.1A scFv and SAM-6 KTA scFV, which has a "KT" instead of an "RP" in VH chain CDR3 of SEQ ID NO:3, respectively, bind to apolipoprotein B100, LDL, VLDL and de-glycosylated LDL (Example 13, pages 97-99). SAM-6 Percevia, which has a change in a framework amino acid of VL also binds to apolipoprotein B100, LDL, VLDL and de-glycosylated LDL. Furthermore, ELISA analysis revealed two additional SAM-6 antibody variants, denoted SAM-6.2.7 (2.7 has an "E" at the first position of VH chain instead of a "Q" compared to SEQ ID NO:1) and SAM-6opti (a VH chain with 4 residues changed compared to SEQ ID NO:3), and the SAM-6 VH chain alone (without VL chain) all bind to apolipoprotein B100 (page 99, first two paragraphs). Additional studies of SAM-6 sequence variants, denoted "optimized scFV dimer," revealed increased affinity for LDL, compared to SAM-6 1.1A scFV and to a SAM-6 optimized scFV monomer (Example 19). Accordingly, Applicants respectfully request consideration of the accompanying binding data, which confirm that VH sequence alone without VL chain, as well as antibodies comprising various sequence variants of VH (SEQ ID NO:3) and VL (SEQ ID NO:1) chains retain target antigen binding activity.

Objection to Claim 37

Claim 37 stands objected to due to an apparent error in the recitation of the term VL instead of VH.

Applicants thank the Examiner for bringing the error to Applicants' attention. As set forth herein, claim 37 has been amended to correct the typographical error. Accordingly, the objection is moot.

I.   REJECTION UNDER 35 U.S.C. §112, FIRST PARAGRAPH

The rejection of claims 27 to 32, 34 to 43 and 48 to 59 under 35 U.S.C. §112, first paragraph, as allegedly lacking written description is respectfully traversed. The grounds for rejection are set forth in the Office Action, pages 6-12.

Applicants respectfully point out that claims 27 to 32, 34, 36 to 43 and 48 to 54 require both light (V_L) and heavy (V_H) chain variable region sequences. Thus, given that these claims require both a light chain (V_L) variable region and a heavy chain (V_H) variable region sequences, all 6 CDRs that confer antigen binding activity are present. Furthermore, certain dependent and independent claims recite the predicted CDRs of heavy (V_H) and/or light (V_L) chain variable region sequences of SEQ ID NOs:1 and 3, which confer antigen binding specificity.

Furthermore, as discussed and corroborated in previously submitted Exhibits B and C, and the attached accompanying confirmatory data from WO 2010/088739, SAM-6 (SEQ ID NO:3) V_H chain alone, without a variable light (V_L) chain, is sufficient to confer binding to target. Furthermore, the data confirm that antibodies comprising sequence variants of both VH and VL chains are able to retain binding to target antigen. Thus, given that heavy (V_H) chain variable region sequence alone is sufficient to confer binding activity, antibodies and polypeptides including a variant heavy chain (V_H) variable region sequence within the scope of the claims could be readily verified for binding activity by routine studies disclosed in the specification or known to the skilled artisan.

As previously pointed out, the VH chain sequence that confers binding to apolipoproteinB containing LDL and oxLDL is disclosed (SEQ ID NO:3). In view of the guidance in the specification and the substantial knowledge and skill in the art at the time of the invention relevant to antibody structure and function, variants that bind apolipoproteinB containing LDL and oxLDL could be produced and identified without undue experimentation. Here, the data in WO 2010/088739 confirm the target antigen binding activity of VH chain alone, and sequence variants of VH and VL chains (SEQ ID NOs:3 and 1), within the scope of the claims.

To corroborate Applicant's position, submitted herewith are pages from PCT WO 2010/088739, which provides concrete examples of variant antibodies made without use of any greater knowledge of antigen identity than is disclosed in the as-filed application. Applicants note that PCT WO 2010/088739 discloses, among other things, variant antibodies, and studies of variant antibody and heavy chain variable region sequence alone binding to apoB-100, VLDL, LDL, or de-glycosylated LDL. Applicants reference these specific studies from the published PCT application as merely examples of studies that confirm that additional knowledge of antigen identity greater than what is disclosed in the subject

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application is not essential to verify antibodies, including sequence variants, that bind to LDL or oxLDL.

Moreover, in regard to the target antigen in LDL and oxLDL, as previously pointed out, antibodies typically bind to proteins due to their strong immunogenicity, and proteins are more likely to be immunogenic than lipids. Here, LDL apparently contains one protein, namely apolipoprotein B100, as stated in Terrlink et al. (J. Lipid Research 45:954 (2004)); namely "each LDL particle contains a single copy of apolipoprotein B-100" (page 955, first column). The specification discloses binding studies of SAM-6 and a control anti-apoB100 antibody to LDL and binding to oxLDL (pages 13-14), which antibodies exhibited identical binding patterns. The claims have also been amended to reflect the binding data by specifying that the LDL and oxLDL contain apolipoprotein B. Additionally, the studies described in WO 2010/088739 previously and submitted herewith are confirmation that numerous sequence variants of SEQ ID NOs:1 and 3, and V_H chain alone, retain binding activity to LDL and oxLDL, which variants are also within the scope of the claims.

Lastly, the claims at issue are distinguishable from the case law cited in the Action. Namely, in *University of Rochester v. G.D. Searle &Co., Inc.* (Fed. Cir. 2002) and in *Ariad Pharmaceutcials v. Eli Lilly & Co.* (Fed. Cir. 2010), there were no working examples of any species disclosed in patents at issue. Likewise, in *Centocor Ortho Biotech Inc., v. Abbott Labs.*, (Fed. Cir. 2011), there was not a single disclosed example of a human antibody or an isolated human antigen in the patent at issue. In *Billups-Rothberg, Inc. v. Assoc. Regional and Univ. Pathologists, Inc.* (Fed. Cir. 2011), again there was not a single disclosed mutation that conferred the genetic defect in the patent at issue. In contrast to all of the aforementioned cases, the specification discloses a working example of an antibody having binding activity. Furthermore, the knowledge and skill in the antibody art at the time of the invention was substantial such that one of skill in the art would have known a number of variants that would be highly likely to retain antigen binding activity. Thus, for at least these reasons, the claims under consideration are distinguishable from the *Rochester, Ariad, Centocor,* and *Billups-Rothberg, Inc.*, cases.

In view of the foregoing and reasons of record, claims 27 to 32, 34, 36 to 43 and 48 to 59 are adequately described under 35 U.S.C. §112, first paragraph. Accordingly, Applicants respectfully request that the rejection be withdrawn.

II.   REJECTION UNDER 35 U.S.C. §102(b)

The rejection of claim 43 under 35 U.S.C. §102(b), as allegedly anticipated by Giles-Komar et al. (WO 2002/12502) is respectfully traversed. The grounds for rejection are set forth in the Office Action, pages 13-14.

Claim 43 has been amended to recite all predicted CDR sequences of SEQ ID NO.:3. Giles-Komar et al. fail to teach or suggest a purified antibody or functional fragment thereof having all predicted CDR sequences of SEQ ID NO.:3. Accordingly, as Giles-Komar et al. fail to teach or suggest each and every element of claim 43, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §102(b).

III.   OBVIOUSNESS-TYPE DOUBLE PATENTING REJECTION

The provisional rejection of claims 27 to 32, 34 to 42 and 48 to 59 under 35 U.S.C. §102(b), as allegedly unpatentable over claims 73, 80, 81, 106 to 112, 115, 116 and 122 to 124 of co-pending application no. 10/579,290 is respectfully traversed. Allegedly, the claims under consideration would have been obvious in view of the conflicting claims of application no. 10/579,290.

Applicants respectfully request that the provisional obviousness-type double patenting rejection be withdrawn since co-pending application no. 10/579,290 has not been allowed, and furthermore, is not in allowable condition nor is in an advanced stage of prosecution, as compared to the subject application. Accordingly, it is appropriate to withdraw the obviousness-type double patenting rejection to this application. Applicants, if appropriate, will file a Terminal Disclaimer in connection with co-pending application no. 10/579,290, should the claims of this co-pending application be indicated to be allowable.

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CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 27 to 32, 34 to 43 and 48 to 59 clearly and patentably define the invention, and respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

For purposes of the Interview, the Examiner is respectfully requested to contact Applicant's representative at (858) 509-4065.

Please charge any fees associated with the submission of this paper to Deposit Account Number 033975. The Commissioner for Patents is also authorized to credit any over payments to the above-referenced Deposit Account.

Respectfully submitted,

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peptide containing the Cys as S-amidomethyl Cys was also found. Thus, only a fraction of this Cys was oxidized.

An ion of [M+H]+ = 1986·1 had a sequence tag that matched A[110-128]K but was 28 Da lighter that expected. From the ETD and LID spectra it was apparent that the peptide was substituted near the C-terminus and a V126A substitution was assigned. This substitution is included in the sequence-coverage diagrams (Figure 23).

Example 9

This example includes a description of how amino acid residues are assigned to the three CDRs in heavy chain variable region and the three CDRs for light chain variable region of SAM-6 and a number of representative variant heavy and light chain variable region sequences. CDR positions are predicted based upon the assignments set forth below and the residue numbering according to Kabat.

HEAVY CHAIN V- domain. According to Kabat numbering, definition is as follows:

CDR-H1

Start:	approx residue 26 (always 4 after a Cys)
Residue before:	always a Cys-xxx-xxx-xxx-
Residue after:	always a Trp, typically WV or WI or WA
Length:	10-12 residues

CDR-H2

Start:	always 15 residues after end of CDR-H1
Residue before:	typically Leu-Glu-Trp-Ile-Gly (LEWIG)
Residue after:	Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (RFT)
Length:	16-19 residues

CDR-H3

Start approx :	always residue 33 after end of CDR-H2 (always 2 after Cys)
Residue before :	always a Cys-xxx-xxx-xxx (typically Cys-Ala-Arg)
Residue after:	always Trp-Gly- xxx-Gly
Length:	3-25 residues

Thus, for **SAM-6 VH: CDR H1 S25-H35; CDR H2 V50-G66; and CDR H3 R98-Y110,** as indicated by the asterisks (*) below. F denotes a framework mutation, and B denotes a mutation in a CDR.

Sequence of SAM-6 VH and representative variants (underlined):

```
                           *****  ******                     *  **********  ******
Percivia      QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
Protein       QVQLVESGGG VVQPGR   L SCAASGFTFS SYAMHWVR       GLEWVAV ISYDGSNKYY ADSVK
1BTA1.1       QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.2 (B)   QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFAI(70)
1BTA1.3 (n/d) RLQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.4 (F,B) QVQLVESGGG VVQPGRSLRL SRAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA1.5 (B)   QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAIHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
1BTA2.2 (F,B) EVQLLESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKDRFTI(70)
1BTA2.7 (F)   EVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)
SAM-6 old (B) QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYAMHWVRQA PGKGLEWVAV ISYDGSNKYY ADSVKGRFTI(70)


                                     **********  ***
Percivia      SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WG
Protein          DNSKNTLY LQMNSLRAED TAVYYCAR
1BTA1.1       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.2       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.3       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA1.4       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAARPFDY WGQGTLVTVS S(121)
1BTA1.5       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA2.2       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
1BTA2.7       SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGRPFDY WGQGTLVTVS S(121)
SAM-6 OLD(KT) SRDNSKNTLY LQMNSLRAED TAVYYCARDR LAVAGKTFDY WGQGTLVTVS S(121)
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LIGHT CHAIN V-domain. According to Kabat numbering, definition is as follows:

CDR-L1

Start: approx residue 24

Residue before: always a Cys

Residue after: always a Trp, typically WYQ or WLQ or WFQ or WYL

Length: 10-17 residues

CDR-L2

Start: always 16 residues after end of L1

Residue before: generally a Ile-Tyr or VY or IL or IF

Length: always 7 residues (except 7FAB)

CDR-L3

Start approx: always residue 33 after end of L2

Residue before: always a Cys

Residue after: always Phe-Gly- xxx-Gly

Length: 7-11 residues

Thus, for **SAM-6 VL: CDR L1 S23-C33; CDR L2 Q49-S55; and CDR L3 Q88-V96** as indicated by the asterisks (*) below. F denotes a framework mutation, and B denotes a mutation in a CDR.

Sequence of **SAM6 VL** and representative variants (underlined):

```
                        *******  ***                       **
Percivia    SYVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
Protein     -YELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
1BTA1.6     SYVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
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1BTA2.1 (F)    QSVLTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)
1BTA2.6 (F)    SYELTQPPSV SVSPGQTASI TCSGDKLGDK YACWYQQKPG QSPVLVIYQD(50)


               *****                        ***  ******
Percivia    SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
Protein     SKRPSGIPER
1BTA1.6     SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
1BTA2.1     SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
1BTA2.6     SKRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCQAW DSSIVVFGGG TKLTVLGQ(108)
```

"Percevia" refers to the sequences used for recombinant IgM antibody expression by the PerC6 cell line (Percivia) PAT-SAM-6. "Protein" refers to the amino acid sequence determined by peptide sequence analysis of the antibody produced by the hybridoma cell line (HAB), as described in Example 8. "SAM-6 old" is heavy chain variable region sequence as set forth herein as SEQ ID NO:15.

Additional amino acid and nucleotide sequences of SAM-6 and representative variant sequences are as follows:

SAM-6 defined full length V domain sequences (variants positions underlined):

SAM-6 1.1 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.2 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFAISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVS
S
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCGCCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.4 VH
QVQLVESGGGVVQPGRSLRLSRAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAARPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCCGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT

ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGCTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.5 VH
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAIHWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATACACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 KT VH (SAM-6 old)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGKTFDYWGQGTLVTVS
S
CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAAAACTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 2.2 VH
EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGACCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCAG

SAM-6 2.7 VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.1A imp VH

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAGACCTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 Opt VH 76/363
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGRPFDYWGQGTLVTVSS

GAGGTGCAGCTGGTCGAGAGCGGGGGAGGCCTGGTCCAGCCAGGGGGATCTCTGAGACT
GAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGCTGGGTGCGCCAGG
CTCCAGGGAAAGGACTCGAATGGGTGGCCGTGATCAGCTACGACGGCAGCAACAAGTAC
TACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCT
GTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCGGTGTACTACTGCGCCAGGG
ACCGGCTGGCCGTGGCCGGCAGACCCTTCGACTACTGGGGCCAGGGCACCCTGGTGACC
GTGTCCTCT

SAM-6 KT imp VH
EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDRLAVAGKTFDYWGQGTLVTVS
S
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGC
TCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGCAATAAATACT
ACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTG
TATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA
TCGGTTAGCAGTGGCTGGTAAAACTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SAM-6 1.6 VL (Used in all "A" scFv constructs, e.g., 1.1A scFv has this light chain sequence.)
SYVLTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSG
SNSGNTATLTISGTQAMDEADYYCQAWDSSIVVFGGGTKLTVLGQ

TCCTATGTGCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCAGCATC
ACCTGCTCTGGAGATAAATTGGGGGATAAATATGCTTGCTGGTATCAGCAGAAGCCAGG
CCAGTCCCCTGTGCTGGTCATCTATCAAGATAGCAAGCGGCCCTCAGGGATCCCTGAGCG
ATTCTCTGGCTCCAACTCTGGGAACACAGCCACTCTGACCATCAGCGGGACCCAGGCTAT
GGATGAGGCTGACTATTACTGTCAGGCGTGGGACAGCAGCATTGTGGTATTCGGCGGAG
GGACCAAGCTGACCGTCCTAGGTCAGA

SAM-6 2.6 VL (Used in all "B" scFv constructs, e.g., 1.1B scFv has this light chain sequence.)
SYELTQPPSVSVSPGQTASITCSGDKLGDKYACWYQQKPGQSPVLVIYQDSKRPSGIPERFSGS
NSGNTATLTISGTQAMDEADYYCQAWDSSIVVFGGGTKLTVLGQ

TCC TAT GAA CTG ACT CAG CCA CCC TCA GTG TCC GTG TCC CCA GGA CAG ACA GCC
AGC ATC ACC TGC TCT GGA GAT AAA TTG GGG GAT AAA TAT GCT TGC TGG TAT
CAG CAG AAG CCA GGC CAG TCC CCT GTG CTG GTC ATC TAT CAA GAT AGC AAG
CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT GGC TCC AAC TCT GGG AAC ACA GCC

ACT CTG ACC ATC AGC GGG ACC CAG GCT ATG GAT GAG GCT GAC TAT TAC TGT
CAG GCG TGG GAC AGC AGC ATT GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC
GTC CTA GGT CAG

SAM-6 Opt VL (Kappa) 123/324
DIQMTQSPSSLSASVGDRVTITCRSGDKLGDKYAWYQQKPGKAPKLLIYQDSKHPSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQAWDSSIVVFGQGTKVEIKR

GAC ATC CAG ATG ACC CAG AGC CCC AGC AGC CTG TCC GCC AGC GTG GGC GAC
AGA GTG ACC ATC ACC TGC AGA AGC GGC GAC AAG CTG GGC GAC AAG TAC GCC
TGG TAT CAG CAG AAG CCC GGC AAG GCC CCC AAG CTGCT G ATC TAT CAG GAC
AGC AAG CAC CCC AGC GGC GTG CCC AGC CGG TTT AGC GGC AGC GGC TCC GGC
ACC GAC TTC ACA CTG ACC ATC TCC AGC CTG CAG CCC GAG GAC TTC GCC ACC TAC
TAC TGT CAG GCC TGG GAC AGC AGC ATC GTG GTG TTC GGC CAG GGC ACC AAG
GTG GAG ATC AAG CGG

Example 10

This example includes a description of producing single chain variable region fragments of SAM-6 antibody heavy and light chain variable regions, and representative variant heavy and light chain variable region sequences, and LDL binding and expression studies of the variants.

The construction is done it two parts, the SM-6 VH domain (1BTA1.1-1.6) and then the SM-6 VL domain (1BTA1.6), which can then be joined together and cloned as a Sfi-BglII fragment into the pPOW expression vector. Both parts can be started at the same time.

Sequencing revealed that there are different SAM-6 VH domains which result in amino acid changes. Thus, several scFv constructs with the different sequences were made. Clone 1BTA1.3 has two changes at the start of the VH domain but they are most likely to be errors in construction. Thus, this clone was not used in scFv construction.

For SAM-6 VH (Part A) (use 1BTA1.1, 1BTA1.2, 1BTA1.5), assemble a 20ul PCR reaction with the following components: DNA template, SAM-6 VH Sfil Fwd primer (565897), SAM-6 VH BamHI Rev primer(565352), 10x reaction buffer, dNTP, water to 20ul, and Phusion polymerase. Cycle 95°C x 1 min, 68°C x 30sec, 72°C x 45 sec. Run 5ul on a 1% agarose gel. A band of ~400bp should be seen. Keep the remainder to assemble the ScFv by joining with the VL domain.

For SAM-6 VL (Part B) (use 1BTA1.6), assemble a 20ul PCR reaction with the following components: 1BTA1.6 DNA template, SAM-6 VL BamHI Fwd primer (565354), SAM-6 VL Bk BglII primer (565565), 10x reaction buffer, dNTP, water to 20ul and Phusion polymerase. Run

22) 620049 VH KT opt II build EVQLVES: 5'

   GGGCACTTGTGATCTCCACCTGTCTTGAATTTTCCATGGCCGAGGTGCAGCTGGT
   GGAGTCT 3'

23) 620113 SM-6 VH domain ApaI rev: 5'

   GAAGACCGATGGGCCCTTGGTGCTAGCTGAGGAGACGGTGAC 3'

24) 620107 HC Rev IgG HpaI New: 5' CTGTCCGTTAACTCATTTACCCGGAGA 3'

25) 633471 EVQLVES Per SM-6 imp seq: 5' GAGGTGCAGCTGGTGGAGTCT 3'

26) 633794 SM-6 IgG VH rev: 5'

   CCGATGGGCCCTTGGTGCTAGCAGAGGACACGGTCACCAGGGTG 3'

27) 633795 SM-6 Opt VH Fwd HC: 5'

   CACCCTGGTGACCGTGTCCTCTGCTAGCACCAAGGGCCCATCGG 3'

28) 633814 SM-6 Opt VLk seq F: 5' GAATTCAGCATGGCCGACATCC 3'

29) 633815 SM-6 Opt VLk seq Rev: 5'

   CACGCTGGGAGCGGCCACGGTCCGCTTGATCTCCACCTTGGT 3'

30) 633861 SM-6 SEQ F: 5' CAAGAACACCCTGTACCTGCA 3'

A summary of nine scFv variants produced and expressed from bacteria using the pPOW vector, and the heavy and light chain variable region sequences they contain are listed below:

SAM-6 1.1A scFv: The 1.1A scFv construct has the same V domain gene sequence as the PAT-SAM-6 IgM (Percivia) gene construct.

SAM-6 1.2A has a single aa change in VH CRD2, and same VL domain as 1.1A

SAM-6 1.4A has 1 framework and 1 VH CRD3 change, and same VL domain as 1.1A

SAM-6 1.5A has 1 VH CDR1 aa change, and same VL domain as 1.1A

SAM-6 2.2A has 2 framework and 1 VH CRD2 changes, and same VL domain as 1.1A

SAM-6 2.7A has 1 framework aa change, and same VL domain as 1.1A

SAM-6 KTA: The main difference between the KTA scFv construct and the 1.1A is that it has 2 amino acid changes in the VHCDR3 binding loop. This binding loop is believed to be the most important region of an antibody as it is in this region that the majority of binding reactions take place. The antigen specificity is attributed to this region. The amino acid sequence in the KTA construct is the same as was originally reported in the first SAM-6 patent application. It is the protein in the SAM-6 "family" that still uses heavy chain variable region amino acid sequence, SEQ ID NO:15.

PAT-SAM-6 (Percivia) IgM: Recombinant antibody produced in PerC6 cells (Percivia). There is an amino acid change in the VL domain framework. This change from a V (Valine) to an E (Glutamic acid) appears to improve protein expression levels by the cells. SAM-6 HAB IgM:

Human hybridoma produced protein (Patrys GmBH, Germany). The gene sequence for this protein differs from the PAT-SM-6 IgM in the VL domain, based on protein sequence by Mass Spec.

SAM-6 1.1B scFv has a the same VH sequence as 1.1A, but a single amino acid change in the VL domain

SAM-6 2.2B scFv has 2 framework and 1 VH CRD2 changes, and single amino acid change in the VL domain

Binding to LDL was used to measure protein expression of the various scFv sequences by bacteria. The data reveal that the strongest signal is observed with the SAM-6 KTA and SAM-6 2.7, indicating that these two variants were produced in higher amounts. The variant residues in each will be combined to incorporate these amino acid changes in an IgG variant of SAM-6.

Example 11

This example includes a description of binding studies showing that SAM-6 diabodies bind to an antigen in conditioned media from A549 cells, and bind to LDL (low density lipoprotein). This example also includes data indicating that certain SAM-6 variants are produced in greater amounts when expressed in cells.

In the ELISA (Figure 24), the two bars to the right show binding of scFv 1.1A and 1.1B to LDL. In brief, LDL is coated onto the plate, then the plate is blocked. SAM-6 diabodies (with FLAG tag at C-terminus) are added (incubated) then the unbound protein is washed away. Another antibody is added that binds to the FLAG tag that is also coupled to HRP (HorseRraddish Peroxidase). This binding can be detected in a colour reaction that is recorded by the ELISA plate reader. An absorbance reading around 1.0 indicates that the proteins are binding.

SAM-6 binds to the cancer cell line A549, so these cells produce target in an accessible form that the antibody binds to, most likely on the cell surface. A549 cells were grown and by day three the cells have formed a confluent layer on the bottom of the tissue culture flask. Spent culture media (now called conditioned media as it now has the A549 cell growth by-products in it, including fetal calf serum– as well as the secreted target protein, but no A549 cells as they are removed by centrifugation) were collected and coated to ELISA wells. The neighbouring (control) well had growth media that never had any cells growing in it. After blocking, the SAM6-diabodies are added and allowed to bind. Unbound protein is washed away and the secondary antibody added for flag tag detection.

included, an LM1 diabody, CM1 diabody, a control VH dimer-which is actually a monomer, a BARB3-diabody, BARB4 diabody, a recombinant PAT-SAM6 450-IgM (produced by PerC6 cells, Percivia), recombinant LM1 41B1-IgM , and SAM6 C8/9 hybridoma IgM. The negative controls are the conditioned media that the A549 cells have been growing in, without the primary antibody and with the secondary antibody. The data show that all of the SAM-6 diabodies bind to an antigen present in the A549 culture supernatent, it is also detected by the recombinant SAM-6 IgM clone 450 (produced by PerC6 cells, Percivia). SAM-6 hybridoma C8/9 gives a very poor signal, but this may be due to protein degradation or hybridoma cell death. This ELISA also shows that the other antibodies tested do not bind to any secreted product in the A549 conditioned media. Only SAM-6 detectably binds to the A549 conditioned media. This ELISA shows that SAM-6 binds to a target in A549 cell conditioned media.

Conditioned media from a second cell line HDFa previously shown not to exhibit cell surface binding to SAM-6 antibodies was studied for binding to SAM-6 antibodies. No binding was detected indicating that this cell line is a good negative control.

Another ELISA was performed on a plate coated with Grp78 protein (from Abnova- cell free protein translation-using wheat germ-non-glycosylated). Binding of recombinant PAT-SAM-6 IgM antibody (clones 450 and 528 produced by PerC6 cells, Percivia) and recombinant SAM-6 1.1A diabody to pure non-glycosylated Grp78 protein was detected. All binding was to pure (non-glycosylated) target Grp78 protein. This data indicates that SAM-6 antibodies and variants bind to grp78 without a carbohydrate moiety. On the second half of the plate binding to conditioned media from A549 cells was detected, whereas negative control LM1 antibodies did not bind to the conditioned media. There was variation in the strength of the signal detected but the target protein may not be uniformly dispersed throughout the sample.

Example 13

This example includes a description of studies showing various forms of SAM-6, including SAM-6 scFv, SAM-6 variants and SAM-6 heavy chain variable region (V_H) alone, without light chain variable region (V_L) bind to an apoB100, protein, LDL, VLDL and deglycosylated LDL.

Antigen specificity: Fresh batches of recombinant protein were made and tested against a panel of proteins to determine specificity for LDL. The ELISAs were repeated several times. Positions of the antigens on the plates were randomized to rule out position effects.

For SAM-6 KTA scFv, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 KTA scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). Note that in these protein samples the soluble B fraction has been removed and only the remaining soluble C fraction is tested. The 3rd time reading are higher as they contain the combined protein level (1CHO4.8) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49). The well contains the antigen, Lewis Y tetrasaccharide bound to HSA(Human Serum Albumin). The positive control (anti-Lewis Y) gave an absorbance reading at A655nm of 0.98 on one ELISA and 0.90 on the other ELISA when binding to its carbohydrate antigen lewis Y.

The strongest binding of SAM-6 KTA scFv is to Apolipoprotein B100. Binding to VLDL, LDL and deglycosylated LDL was also detected.

For SAM-6 1.1A scFv urea solubilized, antigens were coated at 0.5ug/well, volume 50ul/well. Buffer 1 x PBS pH 6.5 Primary antibody SAM-6 1.1A scFv affinity purified (anti-HIS-denatured) soluble C dialysed and added neat (50ul/well) (2BTA46). The 3rd time readings contain the combined protein level (1CHO4.7) from the urea solubilised extraction. The positive control antibody anti-Lewis Y, was anti-FLAG purified and added neat (50ul/well) (2BTA49) gave an absorbance reading at A655nm of 1.2 and 1.0.

Strong binding of SAM-6 1.1A to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 (Percivia), strong binding to Apolipoprotein B100, VLDL, LDL and de-glycosylated LDL was detected.

For SAM-6 HAB produced by human hybridoma (Patrys GmBH, Germany), strong binding to VLDL, LDL and de-glycosylated LDL, was detected but less binding to apolipoprotein B100. The SAM-6 HAB gave variable results in this assay.

In the foregoing studies several different SAM-6 proteins produced in a variety of different formats were compared for their ability to bind to various target antigens, such as LDL (Low Density lipoprotein), VLDL, deglycosylated LDL and apoB100 protein. SAM-6 KTA scFv, 1.1 scFv, PAT-SAM-6 (Percivia) and SAM-6 HAB exhibited various degrees of binding affinity for LDL, VLDL, deglycosylated LDL and apoB100 protein, but not HDL (high density lipoprotein). In this way sequence changes can be linked to function.

Further binding studies to ApoB100 were performed by ELISA analysis. In brief, 250ul of Apolipoprotein B100 (10ug/ml) isolated from low density LDL (purchased from Calbiochem) was coated onto ELISA plates. Plates were blocked, incubated with primary single-chain antibodies (SAM-6.2.7 and SAM-6.opti) and SAM-6 heavy chain variable region (V_H) alone), and then incubated with anti-FLAG-HRP secondary antibody in a total volume of 250ul, and compared to three negative controls (Negative control 1: No coating (blocked), No primary, then anti-FLAG-HRP secondary; Negative control 2: No coating (blocked), then primary, then anti-FLAG-HRP secondary; and Negative control 3: Coated with 10ug/ml ApoB100 (blocked), No primary, then anti-FLAG-HRP secondary).

The results indicated that SAM-6.2.7, SAM-6.opti and SAM-6 heavy chain variable region (V_H) alone) bind to ApoB100 protein.

Example 14

This example includes a description of studies showing that SAM-6 variants can also bind to cancer cell lines A549, BxPC3 and CRL1424.

The following variants were studied: SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia). SAM-6 VHVL opt scFv has an optimized framework with 4 amino acid changes in the VH domain including 25% changes at the nucleotide level. There is one additional change in CDR-H1. The VL domain of SAM-6 VHVL opt scFv is a class switch from lambda to kappa light chain with 40 amino acids changed including 38% changes at the nucleotide level. The free Cys residue was removed from the VL CDR1.

FACS analysis revealed that all of the scFv constructs bind to the three cancer cell lines tested (A549, BxPC3 and CRL1424), but not to the negative cell line HDFa.

Additional studies were performed using confocal microscopy analysis for binding to A549, BxPC3, CRL1424, HT-29, HeLa, and MCF-7 cancer cell lines. The SAM-6 antibodies studied included SAM-6 1.1A scFv, SAM-6 KTA scFv, SAM-6 VHVL opt scFv, SAM-6 HAB, and PAT-SAM-6 (Percivia).

In brief, cells were fixed, the primary antibody was added, then detected with a secondary antibody with a FITC label. The cell nucleus was stained with a DAPI stain that appears blue, and measured in the 600-650 wavelength range. This DAPI image was captured and recorded. If the level of DAPI nuclear stain was kept at a constant level, different studies can be "normalized." The cells were incubated with the primary (test) antibody, and the appropriate labelled secondary

antibody added. In these experiments we used an FITC label that was measured at the maximum intensity observed in the 500-550 wavelength range. The FITC image was recorded. The images were overlays of the DAPI image and the FITC images.

In another set of studies the ability of the proteins to show two active binding sites was studied. In order to see a positive result, the protein would need to bind to the cell surface target antigen on the cancer cell line, with one binding arm, and then the second arm would be required to bind to the human LDL labelled with Alexa 488. Only when these two events occur would a binding event be detected. The Alexa 488 image does not fade and is more stable and we found that it generated a more intense staining image.

The results revealed binding was detected for A549 (Lung), BxPC-3 (pancreatic), HT-29 (colon), HeLa (cervix), MCF-7 (breast) and CRL1424 (melanoma) cells for all five SAM-6 antibodies. Differences were observed in the binding of the SAM-6. In some cell lines it appears that the SM-6 proteins have entered the cell nucleus, when the overlays are done the cell nucleus appears a lighter but brighter blue. None of the five SAM-6 antibodies detectably bind to stomach cancer cell line 23132/93.

Example 15

This example includes a description of additional studies with IgG1 SAM-6 variants, which can also bind to cancer cell line A549.

SAM-6 scFv's were converted into IgG1 using the lambda light chain and the IgG protein expressed in mammalian cells HEK293F. To date, the three different SAM-6 proteins produced in IgG1 format are SAM-6 1.1imp IgG, which contains the 1.1A VH domain with one amino acid change to improve expression, now called 1.1imp. It also contains the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 KT imp IgG, which contains the KTA VH domain with one amino acid change to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinaint IgM construct. SAM-6 opt IgG, which contains the S6 optimised VH domain with four amino acid changes as well as codon optimisation to improve expression and the CH1-CH2-CH3 of IgG1. The light chain is the same lambda light chain used in the recombinant IgM construct. KTA means that the KT VH is paired with the 1BTA1.6 VL domain in the scFv construct.

Culture supernatant was isolated after transient transfection of HEK293F cells. At day 5, cell supernatant with the appropriate IgG1. Binding of SAM-6 1.1imp IgG1, SAM-6 KT IgG1 and SAM-6 opt IgG1 to A549 cells was detected.

Additional FACS studies were performed with HeLa cells with SAM-6 1.1imp IgG1 and SAM-6 Kopti IgG1. Binding of SAM-6 1.1imp IgG1 and SAM-6 Kopti IgG1 to HeLa cells was detected.

Example 16

This example includes a description of studies showing that alterations of amino acid residues in SAM-6 scFvs can increase protein solubility.

The limited solubility of scFv's antibodies, as seen with SAM-6 1.1A, whose Fv sequence is unchanged from parent PAT-SAM-6 IgM, has limited storage and reduced efficiency in trials of the antibody over time. However, as shown in the Table below, with SAM-6 optimized (scFv dimmer) and SAM-6 opt. scFv monomer, codon usage optimization combined with targeted residue changes in the VH domain as well as a new VL domain framework all contribute to improved protein solubility.

Solubility of scFv's in Biological Buffers			
scFv antibody	Concentration from Profinia™ (containing 6M Urea)	Concentraion post dialysis (BCA)	Percent recovery after dialysis
SAM-6 1.1A	1.100 mg/ml	0.174 mg/ml	16%
Optimized dimer	1.200 mg/ml	0.861 mg/ml	72%
PAT-SAM-6 opt monomer	1.300 mg/ml	1.223 mg/ml	94%

The data in the table indicate that SAM6 optimized (diabody) vs SM-6 opt (optimized) monomer have a higher yield than SAM-6 1.1A.

Example 17

This example includes a description of studies showing ELISA binding studies of SAM6-IgM to lipoprotein (LDL) and an apoptosis assay.

PAT-SAM6-IgM shows binding to LDL relative to isotype matched human IgM antibody (Figure 25A). Furthermore, binding of PAT-SAM6-IgM is increased after Cu2+ oxidation of LDL (Figure 25A). Antibody-induced lipoptosis of tumor cells in the presence of differently Cu-oxidized LDL was measured by Cell Death Detection ELISAPLUS. Pancreatic carcinoma cell line BXPC-3 was incubated with PAT-SAM6-IgM and unrelated human IgM isotype control. Amounts of apoptotic cells were determined photospectrometrically at 415 nm (reference λ 490 nm). PAT-SAM6-IgM ability to induce lipoptosis/apoptosis is enhanced in the presence of increased Cu^{2+} oxidized LDL (Figure 25B).

Example 18

This example includes a description of studies showing SAM-6 immunoprecipitation of target antigen, and possibly antigens associated with target antigen, from conditioned media produced by A549 cells.

Immunoprecipitation studies of conditioned media from A549 cells with SAM-6 diabody was performed. The immunoprecipitated portion was fractionated on a10% SDS-PAGE and subsequentely silver stained. As illustrated in Figure 26, SAM-6 diabody binds to several proteins around 110 to 50 kDa, as well as lower molecular weight proteins, present in the A549 cell conditioned media. These proteins may be a SAM-6 target, a target fragment or a protein that is associated with a SAM-6 target. The 30 kDa is presumed to be the SAM-6 diabody.

Example 19

This example includes a description of additional studies showing increased affinity of a particular SAM-6 variant (optimized scFv dimer) for LDL, as compared to SAM-6 1.1A scFv and SAM-6 optimized scFv monomer.
PAT-SAM-6-IgM was previously been shown to bind LDL and induce lipoptosis in cancer cells. Affinity of other PAT-SAM-6 scFv variants to LDL was assessed via ELISA. All variants bind positively to LDL, with PAT-SAM-6 scFv diabody optimized having the greatest affinity.

Example 20

This example includes a description of additional SAM-6 binding studies, and that SAM-6 does not bind to CD55 antigen.
In brief, CD55 antigen (0.5 µg/well) was coated in a volume of 50µl/well. The primary antibody was incubated at 12 µg/ml (0.6 µg/well). Buffer was pH6.5 and for the dilution buffer used was High salt pH8.0. These ELISA studies revealed that SAM-6 does not bind to CD55 antigen.